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AWARD NUMBER DAMD17-97-1-7296

TITLE: Cyclin C Regulation of the Stress Response and Drug Sensitivity in Breast Cancer

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REPORT DATE: August 1998

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1998	Annual (1 Sep 97 - 31 Aug 98)

4. TITLE AND SUBTITLE
Cyclin C Regulation of the Stress Response and Drug Sensitivity in Breast Cancer

5. FUNDING NUMBERS
DAMD17-97-1-7296

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for Public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Disseminated malignancies are commonly treated with cytotoxic agents (e.g., chemotherapy, radiation) which target the unregulated growth associated with tumors. However, many of these procedures have proven unsuccessful due in part to the acquired resistance of cancer cells to these regimens. Mounting evidence suggests that one underlying mechanism by which malignancies are protected from cytotoxic agents is through aberrant activation of a pathway generally referred to as the "stress response". Using a genetic approach in yeast, we have identified a new C-type cyclin (*UME3*) that, when deleted, allows the inappropriate expression of the HSP70 family member SSA1. Several pieces of data suggest that the human cyclin C (cycC), which exhibits nearly 40% identity to the yeast gene, may also be involved in regulating the stress response. First, cycC co-localizes with the human RNA polymerase suggesting a role for this cyclin in transcriptional regulation. Second, when expressed in yeast, cycC is rapidly destroyed in cultures exposed to elevated temperatures. Finally, we have mapped cycC to a region of the genome (6q21) that is frequently deleted in breast tumors. This proposal will explore the relationship between cycC activity, the stress response and drug sensitivity.

14. SUBJECT TERMS	15. NUMBER OF PAGES
cyclin/stress response/drug resistance	6
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 754-01-280-5500

Standard Form 298 (Rev 2-89)

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Randy Strucks
Principal Investigator's Signature

6/26/98
Date

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Progress Report

Human cyclin C displays a high degree of homology to the *S. cerivisie* Ume3p protein. We have shown that Ume3p is degraded in response to environmental stresses such as heat, ethanol and H_2O_2. In addition, the human cyclin C is also destroyed in response to heat in yeast. Therefore we designed experiments to test whether the human cyclin C behaves similarly in mammalian systems. This was accomplished by analyzing its behavior in an assortment of mammalian cell lines.

Cell lines initially investigated were 293T (human kidney, epithelial-like morphology), Vero (monkey kidney, fibroblast morphology), COS-1 (monkey kidney, fibroblast morphology), MDA-MB-231 (human breast, epithelial-like morphology), MCF7 (human breast, epithelial-like morphology) and HeLa (human cervix, epithelial-like morphology). HeLa and MCF7 cell lines were chosen for the majority of experiments.

Antibodies directed against Hsp70 (Stressgen) where used to measure the induction of the stress response pathway. The anti-Hsp70 used in this study is specific for the stress-inducible from of Hsp70. Cyclin C levels where measured using antibodies directed against the tagged portion of a cyclin C construct generated in this study. After transfection into the cell line of interest, cells were heat shocked (42° or 45°C). No detectable variation in cyclin C levels where observed following heat shock. This observation could be due to deviations in our experimental system (e. g., the presence of the epitope tag or the high expression levels produced by the viral promoter). Therefore, an alternative approach was pursued. Antibodies directed against cyclin C were obtained from Dr. Emma Lees at DNAX. These antibodies allowed the detection of the endogenous cyclin C protein.

The heat shock response was again analyzed but this time using antibodies directed against the native cyclin C. Neither HeLa or MCF7 cell lines exhibited any significant change in cyclin C levels during heat shock. This is in contrast to the Ume3 C-type cyclin studies in yeast where the yeast homologue is rapidly degraded. The regulation of cyclin C was then examined under conditions of alcohol (ethanol) or oxidative (H_2O_2) stress. Again, in contrast to the Ume3p studies, no significant change in cyclin C levels were detected.

Due to chromosomal abnormalities frequently found in immortal cell lines, we next examined two primary cell sources available to us. Primary cells obtained from Xenopus oocytes and mice neurons were first tested for cross reactivity against the human cyclin C antibody. While Xenopus exhibited no cross reactivity, mouse extracts cross-reacted with an apparent mouse cyclin C. Preliminary heat shock studies using mouse neurons have failed to exhibit any changes in cyclin C levels during stress.

In order to determine if the same degradation machinery that is present in yeast is also present in the mammalian systems, the yeast Ume3 C-type cyclin has been transfected into MCF7 cells. These cells are to be heat shocked and the Ume3p levels analyzed. Stable transfected MCF7 cell lines containing either an HA tagged

human cyclin C or a myc tagged Ume3p have been generated. These lines will be used in the further dissection of the stress response in mammalian cells.

The lab is also currently pursuing the study of primary cell lines to help further elucidate cyclin C's response to stress in mammalian systems.